

MORPHOLOGICAL AND GENETIC EXAMINATION OF PHENOTYPIC VARIABILITY IN THE TROPICAL SPONGE *ANTHOSIGMELLA VARIANS*

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Hill, M.S. 1999 06 30: Morphological and genetic examination of phenotypic variability in the tropical sponge *Anthosigmella varians*. *Memoirs of the Queensland Museum* 44: 239-247. Brisbane. ISSN 0079-8835.

Sponges commonly exhibit phenotypic variation in response to a heterogeneous environment. Determining the ecological causes and understanding the evolutionary consequences of this variation is a primary goal of biologists. Three ecotypes of the common Caribbean sponge *Anthosigmella varians* (Demospongiae: Hadromerida: Spirastrellidae) have been identified, and they provide a model system for exploring morphological variation. *Anthosigmella varians* forma *incrustans* is an encrusting bioeroder located on fore- and back-reef environments at depths ranging from 3->30m; *A. varians* forma *varians* is an irregularly lobate branching ecotype found in shallow (<1m) water near-shore; and *A. varians* forma *rigida* is a branching form found in sympatry with *incrustans* but restricted to shallower depths. In this paper, a detailed examination of ecologically important characters (e.g. tissue strength, skeletal properties, distribution) for all 3 morphs is presented. Using allozyme electrophoresis, fixed differences at two loci were discovered indicating potential reproductive isolation between branching (*rigida* and *varians*) and encrusting (*incrustans*) morphotypes. Results from a transplantation experiment indicate that sediment load may be an important factor in branch production. Sedimentation may also explain the competitive aggressiveness of *incrustans* which is often found growing over coral species (i.e. *A. varians* grows over corals to gain access to CaCO₃ skeletons which are typically low sediment zones). It is proposed that *rigida* populations can exist on the reef due to the production of a spicule- and collagen-rich cortex that provides a structural defense against predators. It is suggested that wave energy may have less important effects on branch production in *A. varians* than either predation or sedimentation. □ *Porifera, phenotypic plasticity, spongivory, population structure, cryptic species, Anthosigmella varians.*

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Sponges present a unique challenge to our understanding of evolutionary processes in the sea. This ancient group of metazoans has few traits that are taxonomically useful for distinguishing closely related species, and those that are employed in assigning taxonomic positions can often be modified by environmental parameters. For example, wave energy has been demonstrated to affect gross morphological characteristics of intertidal sponges (Palumbi, 1984, 1986) and changes in spicule size and shape can often reflect the influence of extrinsic factors (e.g. Jones, 1970, 1971). Thus, there is a danger of misinterpreting the evolutionary history of this metazoan group. Determining when morphological variation in sponges represents phenotypic plasticity or true speciation is a significant challenge.

Several investigators have shown that morphological variants previously included

within a single sponge taxon represent distinct species (Sole-Cava & Thorpe, 1986, 1990; Sole-Cava et al., 1992; Bavestrello & Sara, 1992; Barbieri et al., 1995). These studies indicate that we know little about sponge diversity, and ultimately, evolutionary processes in the ocean. These studies also highlight how poorly we understand the importance of phenotypic variation in the ecology and evolution of sponges. When faced with morphological varieties of a species, two questions must be addressed. First, are the phenotypic variants reproductively isolated? This can be inferred by examining the genetic constitution of populations. Second, if the variants are part of an interbreeding population, are environmental factors responsible for the observed variation, and if so, which are most important? These questions are of particular concern on tropical coral reefs since ongoing debate concerns the relative importance of equilibrial and nonequilibrial processes in the

maintenance and origin of diversity (Sale, 1994; Knowlton & Jackson, 1994a, b). If environmental parameters are highly predictable, then niche partitioning via habitat specialisation may be a common product of evolution on coral reefs (Knowlton & Jackson, 1994a; Palumbi, 1994). If, however, larvae settle randomly into diverse habitats, or disturbance frequencies differ among habitats, then phenotypic plasticity may evolve (e.g. Lively, 1986).

Sponges present a highly tractable system for examining ecological conditions necessary for the evolution of phenotypic plasticity. They also provide a model for identifying factors important in the evolution of reproductive isolation (i.e. speciation) between morphologically divergent populations. Here, I present an analysis of morphological and genetic differences among morphotypes of the common Caribbean sponge *Anthosigmella varians* (Demospongiae: Hadromerida: Spirastrellidae). In addition, I discuss the roles that phenotypic plasticity and habitat specialisation may have played in the evolution of this species.

NATURAL HISTORY. *Anthosigmella varians* (Duchassaing & Michelotti) is a common sponge of Caribbean coral reefs (Wiedenmayer, 1977; Vicente, 1978). This sponge harbors intracellular dinoflagellate zooxanthellae, bores into calcium carbonate structures (Illl, 1996a) and exhibits two distinct morphologies: branching and encrusting (Wiedenmayer, 1977). It is included in the diets of angelfish (Randall & Hartman, 1968; Hourigan et al., 1989) and at least some parrotfish species (pers. obs.; Dunlap & Pawlik, 1996; Wulff, 1997). Taxonomists recognise two morphotypes: an encrusting growth form (forma *incrustans*) and an amorphous, irregularly lobate, branching growth form (forma *variens*), and consider these to be ecophenotypes based on their occurrence in different habitats (Wiedenmayer, 1977). *Anthosigmella varians* forma *incrustans* (hereafter referred to as *incrustans*) is conspicuous on fore- and back-reefs while *A. variens* forma *variens* (hereafter referred to as *variens*) is typically found in shallow, lagoonal areas.

In the Florida Keys, USA, *variens* is found close to shore on both bay and ocean sides of many islands; *incrustans* is only found on the reefs which run parallel to the islands approximately 8km from shore (Fig. 1). Wave energy has been proposed as the factor responsible for this distribution: *variens* is presumed to be unable to handle the strong currents and periodic

heavy wave action that open reefs receive (Vicente, 1978). During this study, a number of branching morphs sympatric with encrusting morphs were encountered on both Tennessee and Alligator fore-reefs in the Florida Keys (as well as Molasses Reef; J. Pawlik, pers. comm.) (Fig. 1, Table 1). This morph has lobate branches like *variens* but is easily distinguishable due to its much stiffer skeletal construction. It was often found less than a meter from *incrustans* individuals. I refer to this morph as *A. variens* forma *rigida* (hereafter as *rigida*).

MATERIALS AND METHODS

There were three components to this study: 1) comparison of morphological and ecological characteristics of the three morphs of *A. variens*; 2) transplant experiments to assess the effect of sedimentation on morphological variation in *variens* and *rigida*; and 3) allozyme analysis, used to estimate genetic relatedness among *A. variens* morphs.

MORPHOLOGICAL CHARACTERISTICS.

External. The following external features were measured in situ; surface area of attachment, number of branches, branch length, maximum height, mound height and tissue strength. Sample sizes for all parameters are listed in Table 1. Surface area of attachment was estimated using a 1m² quadrat marked off in 25cm² increments. Branch length was measured as the distance from tip to node. Maximum height was measured from the highest point of the sponge perpendicular to the substratum. Mound height was measured from the substratum to the highest point on the mounding portion of branching individuals.

The method for determining tissue strength was adapted from Palumbi (1984). A barbless hook, attached to a spring scale, was embedded to a depth of 0.5cm into the surface tissue of an individual. Care was taken to ensure that there was no foreign material in the sponge tissue. The spring scale was pulled perpendicularly until the hook tore free, and the maximum force required to extract the hook was recorded. Three pulls were averaged for each sponge, sample sizes for each morph are shown in Table 1. One-way ANOVA was used to compare morphotypes (Zar, 1984).

Internal. Several internal characters were also compared among morphs. Cortex thickness was measured for seven individuals from each morph, 10 measurements were averaged for each individual. The cortex of a sponge is defined as

TABLE 1. Distribution and morphological characteristics of three morphotypes of *Anthosigmella varians* in the Florida Keys. Values represent means ($\pm$ SE); sample sizes are shown. Values sharing a superscripted letter are not significantly different at $P > 0.05$.

Parameter	<i>incrustans</i>		<i>varians</i>		<i>rigida</i>	
	Reef	n	Bay/near island	n	Reef	n
Distribution	Reef		Bay/near island		Reef	
Depth (m)	8 - 27		1 - 3		8 - 13	
Number of branches	0a	48	2.95 (0.3) ^b	55	1.92 (0.62) ^c	13
Branch length (cm)	n/a	-	10.37 (0.51) ^a	161	4.36 (0.79) ^b	25
Mound Height (cm)	n/a	-	3.47 (0.28) ^a	38	3.38 (0.64) ^a	13
Maximum Height (cm)	n/a	-	12.95 (1.22) ^a	55	6.67 (1.8) ^b	13
Area of attachment (cm ²)	1430.6 (300.5) ^a	48	68.1 (14.4) ^b	32	36.3 (4.79) ^c	13
Tissue strength (N)	4.9 (0.36) ^a	28	2.3 (0.14) ^b	61	>10 ^c	8
Cortex thickness (mm)	0.99 (0.14) ^a	7	n/a	-	5.7 (1.33) ^b	7
Open Space in choanoderm	10.1% (1.8) ^a	7	32.7% (2.1) ^b	7	21.1% (1.9) ^c	7
Spicule conc. (mg cm ⁻³)	52 (2) ^a	7	112 (5) ^b	8	168 (11) ^c	5
Zooxanthella cm ⁻² (H 10 ⁶)	0.86 (0.053) ^a	8	1.37 (0.092) ^b	13	1.03 (0.82) ^{a,b}	3
Wet weight (g cm ⁻³)	1018 (104) ^a	6	833 (39) ^a	10	759 (105) ^a	4
Sponge biomass (g cm ⁻³)	80.8 (8.2) ^a	6	100.8 (3.8) ^a	10	97.2(7.6) ^a	4
Spicule (mg cm ⁻³)	70.5 (13.9) ^a	6	132.6 (15.0) ^b	10	199.7 (17.4) ^c	4
Calcareous debris (g cm ⁻³)	553.9 (66.8) ^a	6	-2 (7.3) ^b	10	-3 (1.1) ^b	4

the layer of ectosome consolidated by a distinctive skeleton (Kelly-Borges & Pomponi, 1992). Measurements were made from the surface along the growth axis to the point where the cortex met the choanosome. Zooxanthellae densities were also compared among morphs. Densities were quantified using methods described in Hill (1996a). Spicule concentrations were estimated as follows: samples of known volume were dissolved in nitric acid until all tissue was removed; the resulting solution was washed several times with ddH₂O and then dried at 60°C for 48hrs. Results were reported as mg of spicule cm⁻³ of sponge tissue.

To determine if there were potential differences in pumping capabilities among morphs, I estimated the percentage of open space in the choanosome of each morph. Five estimates were taken at different locations within the choanosome of a single individual, and these were averaged to give one value per sponge. Seven individuals were measured from each morph. Percentages were arcsin transformed before they were compared using one-way ANOVA (Zar, 1984).

Differences were compared among morphs in the relative content of spicules, biomass and calcareous debris. Wet weights and volumes were recorded for individuals from each morph. Sample sizes are shown in Table 1. Samples were placed in crucibles and weighed after drying for

48hrs at 60°C. Crucibles were then placed in a furnace for 6hrs at 450°C. This procedure removed sponge tissue but left behind spicules, calcareous debris and ash. Ash was washed from crucibles with ddH₂O, and crucibles were then dried for 48hrs at 60°C. After weighing, crucibles were treated with 5% HCl (which removed all CaCO₃), washed with ddH₂O, dried for 48hrs at 60°C and weighed. At this point, all that remained in the crucibles was spicular material.

The distribution and concentration of collagen was compared among morphotypes using the Mallory-Heidenhain's connective tissue stain (Humason, 1979). Immediately after collection, samples were fixed in Bouin's solution. Fixed samples were run through a series of dehydration steps prior to embedding. Samples were placed in paraplast embedding media, allowed to harden, and then sectioned. After staining, sections were placed on slides for viewing.

Spicule measurements were conducted following methods described in Palumbi (1986) with the following modifications. Spicules from 10 *varians* individuals, 6 *incrustans* individuals and 4 *rigida* individuals were cleaned of tissue using concentrated nitric acid (Kelly-Borges & Pomponi, 1992). Samples were then washed with ddH₂O and dried. Spicules were placed on glass slides and a cover slip was mounted over the preparation. Measurements of spicules were

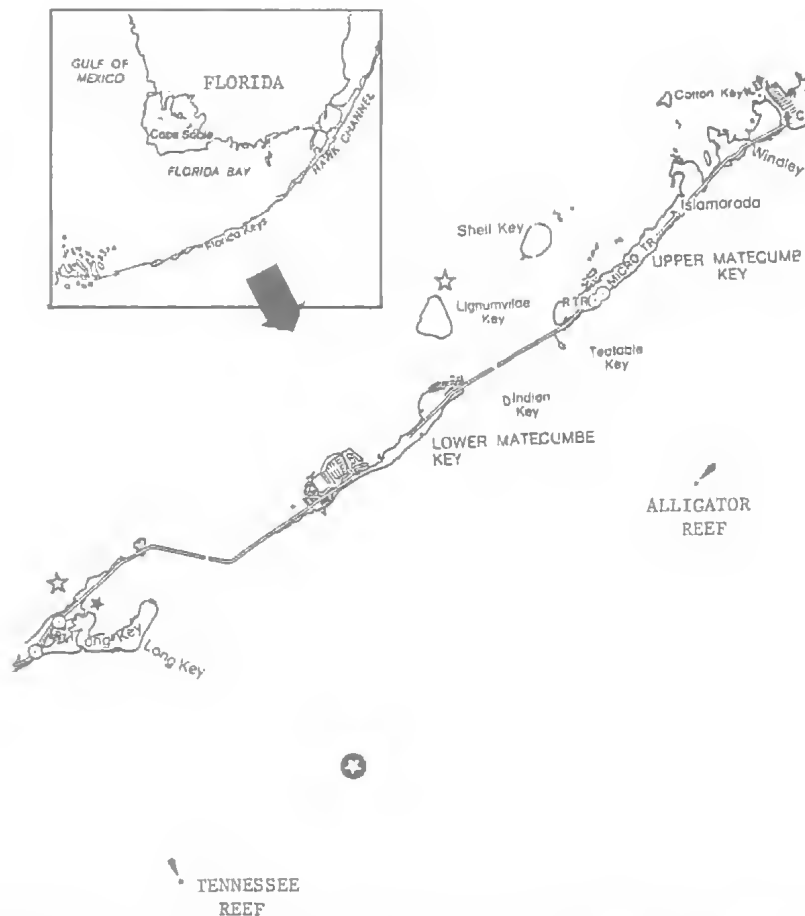


FIG. 1. Map of Florida's Middle Keys; all collections for allozyme analysis were made in areas with stars as well as on Tennessee and Alligator reefs. Reef habitats (i.e. reefs between, and including, Tennessee and Alligator reefs) harbored *incrusters* and *rigida* populations, while near-shore areas harbored *varians* populations.

made from digitised images. Total length and head and shaft widths of subtylostyles were measured for 50 spicules from each individual from each morph. A qualitative assessment of anthosigma shape was also performed at this time. Comparisons of spicule size and shape were made using one-way ANOVA (Zar, 1984).

TRANSPLANT EXPERIMENTS. To examine the effect of sedimentation on morphological variation, several *varians* and *rigida* individuals were transplanted onto platforms that were raised above heavily sedimented substrata. Transplanted *varians* individuals ($n=8$) were placed on the upper surface of cinder blocks at a depth of <1 m. These sponges were monitored for 6 months. Morphological changes were monitored in 10 *rigida* individuals that were affixed to ceramic tiles ($15\text{cm} \times 15\text{cm}$) by fishing line. The ceramic

tiles were placed above the substrata and were attached to wooden planks that had been cemented to the reef. Both of these transplantations provided low sediment zones that represented uncolonised (i.e. competitor-free) space.

ALLOZYME ANALYSIS. Samples of each morphotype were collected in July, 1996. Sympatric individuals of *incrusters* and *rigida* were collected on both Alligator and Tennessee reefs at a depth of 8 m (Fig. 1). *Anthosigmella varians* forma *varians* individuals were collected from the bayside of Long Key and Lignumvitae islands at a depth of 1 m (Fig. 1). Small sections ($<5\text{cm}$ in length) were sampled from each individual and transported to the Key Largo Marine Research Laboratory. Within 4 hrs of collection, portions of the endosome were removed from each specimen and frozen in liquid

nitrogen. The possibility of banding artefacts due to zooxanthellae was reduced by avoiding the pinacosome (general location of the symbiont). Samples were stored at -80°C after shipping.

Samples were processed by grinding a small ($<15\text{mm}^3$) piece of sponge with a stainless steel rod in a 0.1% aqueous solution of β -mercaptoethanol with NADP (2mg ml^{-1}) added. Grinding wells were kept on ice at all times. Approximately $0.25\mu\text{l}$ of the supernatant was applied to cellulose acetate gels. All samples were run at 200V for 15mins in a Tris-glycine buffer (pH 8.5). Staining procedures followed those described in Richardson et al. (1986) and Hebert & Beaton (1993).

Preliminary screening of 30 enzyme systems revealed seven that showed good resolution and could be reliably scored. The enzyme systems employed in this study are shown in Table 2. Genotypes at eight loci were scored directly by examining stained gels. At each locus, alleles were distinguished based on the relative mobilities of their products.

Abbreviation: EC, Enzyme Commission number.

RESULTS

GROSS MORPHOLOGY. There were significant differences in the majority of morphological characteristics measured for the three morphs (Table 1). The most conspicuous trait that differed among morphs was the presence of branches: *incrusters* lacked branches entirely, but covered far greater surface area on average than either *rigida* or *varians*. Hook extraction force (i.e. tissue strength), cortex thickness and spicule concentration were greatest in *rigida*. Zooxanthellae density, number and height of branches, as well as mound height were greatest in *varians*. The percentage of open space in the choanosome was greatest in *varians* and lowest in *incrusters*. The non-transformed percentages are shown in Table 1, but significance values were based on arcsin transformed data.

Standardising ratios of biomass, spicule weights and calcareous debris to dry weights revealed interesting trends. Greater than 75% of *incrusters* tissue was composed of calcareous debris whereas there was no evidence of CaCO_3 within the tissues of either *rigida* or *varians* (Table 1). Residual material that could not be washed out with ddH_2O after the acid treatment resulted in slight increases in crucible weights for both *rigida* and *varians* (hence the negative values in Table 1). Sponge biomass represented

approximately 43% of dry weight for *varians* compared to only 11% for *incrusters*. However, wet-weights were statistically indistinguishable among *varians*, *rigida* and *incrusters*. Spicule concentrations standardised to dry weight showed the following trend: *rigida* > *varians* > *incrusters*. These values matched spicule concentrations measured previously (Table 1).

Collagenous fibers stained bright blue using the Mallory-Heidenhain's connective tissue stain. Collagen was densely concentrated within the cortex of both *incrusters* and *rigida*, but was more extensive in *rigida* since the cortex was thicker in this morph (Table 1). Collagen content in *varians* was negligible and was widely scattered throughout the choanosome.

There were no differences in the lengths of subtylostyles in any morph (Fig. 2), but *varians* had significantly wider megascleres (i.e. head and shaft; Fig. 2). There were no significant differences in widths between *incrusters* and *rigida*. The subtylostyles of *incrusters* were more curvaceous than *rigida* or *varians*. Sigmata, termed anthosigmas in this group, typically had a single bend (i.e. bow shaped) in *incrusters* and *rigida* but often had two or more bends (i.e. sigma shaped) in *varians*. Samples taken from the cortex and choanosome of *rigida* appeared to have no differences in lengths or widths.

TRANSPLANT EXPERIMENTS. Both *varians* and *rigida* began to encrust the surfaces on to which they were transplanted. The manner of encrustation was the same. A thin sheet proceeded to take over the unoccupied area spreading from the base of the branch that had been attached to the substratum. This growth was relatively rapid and may have represented tissue reorganisation rather than true increases in biomass (for example, see Jackson & Palumbi, 1979).

ALLOZYME ANALYSIS. Heterozygotes at loci Glucose-6-Phosphate Isomerase (EC 5.3.1.9; *Gpi*), Malate Dehydrogenase (EC 1.1.1.37; *Mdh-1*) and 6-Phosphogluconate Dehydrogenase (EC 1.1.1.44; *6Pgdh*) all showed 3-banded zymograms typical of a dimeric enzyme. Heterozygous individuals exhibited a multi-banded zymogram for Fumarate Hydratase (EC 4.2.1.2; *Fum*) as would be expected for a tetrameric enzyme; individuals assumed to be heterozygous at Malic Enzyme (EC 1.1.1.40; *Me*) (a suspected tetrameric enzyme) exhibited a smear. No heterozygote was detected at Arginine Kinase (EC 2.7.3.3; *Ark*) or Creatine Kinase (EC

TABLE 2. Allele frequencies for the 8 enzyme loci scored in this study categorised by morphotype of sponge. *Incrustans* = encrusting morph, *varians* = bay branching morph and *rigida* = ocean branching morph. N = number of individuals used for each enzyme system.

Locus	EC #	Allele	<i>incrustans</i>	<i>varians</i>	<i>rigida</i>
Ark	2.7.3.3	1	0.11	0	0
		2	0	1.0	1.0
		3	0.22	0	0
		4	0.67	0	0
		N	9	8	9
Ck	2.7.3.2	1	0.22	1.0	1.0
		2	0.78	0	0
		N	9	8	9
Fum	4.2.1.2	1	0.78	1.0	1.0
		2	0.22	0	0
		N	9	8	9
Gpi	5.2.1.9	1	0	0.03	0
		2	0.14	0.40	0.09
		3	0.86	0.57	0.91
		N	14	15	11
Me	1.1.1.40	1	0.67	0	0
		2	0.33	0	0
		3	0	1.0	1.0
		N	9	7	6
Mdh1	1.1.1.37	1	0.28	1.0	1.0
		2	0.72	0	0
		N	9	8	9
Mdh2	1.1.1.37	1	0	0.75	0.94
		2	0.11	0	0
		3	0.22	0.25	0.06
		4	0.67	0	0
		N	9	8	9
6Pgdh	1.1.1.44	1	0.33	0.5	0.25
		2	0	0.08	0
		3	0.17	0	0.67
		4	0.28	0.25	0.08
		5	0.22	0.17	0
		N	9	8	9

2.7.3.2; *Ck*) loci. Neither *varians* nor *incrustans* populations had heterozygotes at the *Mdh*-2 locus. Encrusting populations appear to be genetically distinct from both branching morphs. There were fixed differences at *Ark* and *Me* loci (Table 2). In addition, there were large differences between encrusting and branching morphs in frequencies for the following loci: *Ck*, *Fum*, *Mdh1* and *Mdh2*. However, allelic frequencies for *Gpi* were more similar in oceanside populations.

DISCUSSION

Anthosigmella varians represents a morphologically diverse species with substantial phenotypic differences among the three morphotypes (Table 1). The distribution of the three morphs (Table 1) indicates that *varians* and *rigida* individuals prefer shallower and more sediment-laden habitats than *incrustans*. Despite significant differences among morphs, the presence of branches is the most useful diagnostic trait available in the field: *rigida* and *varians* have branches while *incrustans* assumes only an encrusting form.

Preliminary grafting experiments demonstrated that all three morphs were capable of attaching to one another, but connections between *rigida* and *varians* were strongest (unpublished results). Although sample sizes are small, genetic analysis supports this distinction since *rigida* and *varians* populations were fixed for alleles at two loci that were not present in the *incrustans* population. Any conclusions about reproductive isolation must be tentative, however, given the absence of heterozygotes at the loci *Ark*, *Ck* and *Mdh*-2. For this reason, the potential reproductive isolation in *A. varians* is being further explored using larger sample sizes and DNA-based molecular techniques which provide access to a larger number of polymorphic loci.

Transplant experiments indicated that sediment may be responsible for the branching phenotype since *varians* and *rigida* individuals that were placed on sediment-free substrate (in both the Florida Bay and reef) began to encrust. Sedimentation rates are highest in the shallow lagoonal areas where *varians* is found while on the reef sedimentation rates appear to decrease with depth (J. Schmerfeld, pers. com.). Although correlative at this stage, this information supports the claim that branching is a response to sediment load.

There is a strong positive correlation between tissue strength and cortex thickness (Table 1); *varians* lacks any sort of cortex, *incrustans* has a well defined but relatively thin cortex, and *rigida* has a thick cortex. The cortex was shown to be collagen rich (with Mallory-Heidenhain stain), and it is probable that the collagen accounted for the dramatic increases in tissue strength in *rigida*. The observed differences among the three morphs may be due to a number of environmental parameters. Wave energy has been hypothesised to prevent *incrustans* from producing branches (Vicente, 1978). Another possibility that has not been considered to date is that predation causes

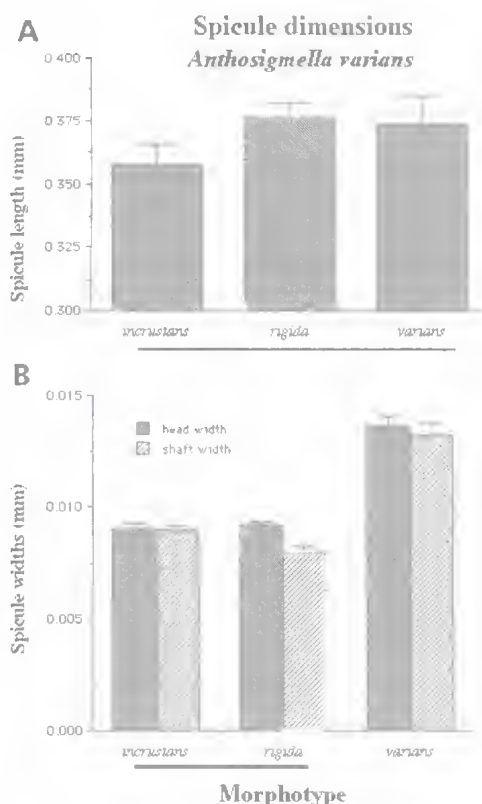


FIG. 2. Measurements of total length and widths of head and shaft in subtylostyles of *A. varians* morphs. Lengths and widths of 50 randomly chosen spicules were averaged for each individual examined. The number of individuals used were: *incrustans* = 6, *rigida* = 4 and *varians* = 10. Histograms represent means ($\pm$ SE); bars connected by an underline are not significantly different at $P > 0.05$ using one-way ANOVA and Tukeys' multiple comparisons test.

increases in collagen synthesis in the cortex. Several lines of evidence suggest that predators, and not wave energy, influence tissue strength and branch production in *A. varians*. For instance, numerous *rigida* individuals were found on the high wave energy reef crest. Qualitative support for this hypothesis came when *rigida* and *incrustans* individuals were cut open to expose the choanosome. Angelfish immediately consumed large quantities of the interior portions of these individuals while completely avoiding the cortex. Caging and transplant experiments indicate that predation is probably more important than wave energy (Hill, 1996b), but the influence of wave energy on collagen production cannot be ruled out.

Encrusting and branching morphs play very different roles in coral reef communities. Over 40% of a surveyed *incrustans* population ($n=48$) was involved in competitive encounters while none of the branching morphs were ever observed growing over corals (Hill, 1996b). Furthermore, by occupying large areas of reef, *incrustans* indirectly affects invertebrate recruitment by usurping space that could be used for successful settlement (Table 1; Vicente, 1978). Given that *incrustans* appears to devote less tissue volume to pumping activities (i.e. fewer choanosomal open spaces; Table 1), it seems that *rigida* and *varians* individuals should have a larger impact on bacterioplankton communities than *incrustans*. Finally, *incrustans* penetrates deeper into carbonate structures than either *varians* or *rigida*. Neither *varians* nor *rigida* devoted as high a percentage of their biomass to boring activities (Table 1), and sponge-produced sediment is often seen on the surfaces of *incrustans* individuals indicating active boring. These observations indicate that *incrustans* plays a larger role in bioerosional processes than either *rigida* or *varians*.

If the branching and encrusting morphs of *A. varians* are truly reproductively isolated populations, then the speciation process must be explained (see discussion of Sarà, 1990). Two distinct, but not exclusive, schools of thought have been adopted by biologists to explain how the great diversity of tropical coral reefs has originated and is maintained. Many attribute observed patterns of diversity to non-equilibrium processes such as disturbance and chance (Sale, 1977, 1988; Connell, 1978, 1979; Hughes, 1989; Doherty & Fowler, 1994; Aronson & Precht, 1995). Hypotheses involving equilibrium processes, such as niche diversification, have recently received increased attention (e.g. Jackson, 1991; Knowlton & Jackson, 1994a). However, there is no clear non-equilibrium mechanism proposed to explain the origin of diversity on coral reefs (Sale, 1988). Although they are assumed to play a major role (Knowlton, 1993; Knowlton & Jackson, 1994a), it is unknown how important habitat specialisation and niche diversification have been in the origin of diversity. The results presented here suggest that niche diversification may have played a role in the separation of *incrustans* from *rigida* and *varians*. That is, *varians* and *rigida* populations are capable of utilising sediment laden environments that *incrustans* is unable to utilise.

Recent research has demonstrated that Poriferan diversity may be greater than indicated by current classifications. For example, Boury-Esnault et al. (1992) measured genetic divergence among sympatric morphotypes of the Mediterranean sponge *Oscarella lobularis* using protein electrophoresis. They found fixed differences at seven loci among sympatric morphotypes indicating a significant reduction (or cessation) in gene flow among populations. The results presented here, in addition to several other studies asking similar questions (e.g. Boury-Esnault et al., 1992; Stobart & Benzie, 1994; Barbieri et al., 1995), suggest that niche partitioning may be a very important diversifying process for tropical sponges.

It is clear that examination of Poriferan diversity, especially morphologically diverse species, is essential if we are to understand evolutionary trends in the simplest metazoans. The small number of informative taxonomic traits in this Phylum has hindered interpretation of patterns and processes. Greater attention must be focused on elucidating microevolutionary processes operating in marine environments, and identifying barriers to gene flow should be a priority. Sponges such as *A. varians* provide a model system to address these questions.

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